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Clinical chorioamnionitis at term VIII: a rapid MMP-8 Test for the identification of intra-amniotic inflammation

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Abstract

Objective—Clinical chorioamnionitis is the most common infection/inflammatory process diagnosed in labor and delivery units worldwide. The condition is a syndrome that can be caused by: 1) intra-amniotic infection; 2) intra-amniotic inflammation without demonstrable microorganisms (i.e., sterile intra-amniotic inflammation); and 3) maternal systemic inflammation that is not associated with intra-amniotic inflammation. The presence of intraamniotic inflammation is a risk factor for adverse maternal and neonatal outcomes in a broad range of obstetrical syndromes that includes clinical chorioamnionitis at term. Although the diagnosis of intra-amniotic infection has relied on culture results, such information is not immediately available for patient management. Therefore, the diagnosis of intra-amniotic inflammation could be helpful as a proxy for intra-amniotic infection, while results of microbiologic studies are pending. A rapid test is now available for the diagnosis of intraamniotic inflammation, based on the determination of neutrophil collagenase or matrix metalloproteinase-8 (MMP-8). The objectives of this study were to 1) evaluate the diagnostic indices of a rapid MMP-8 test for the identification of intra-amniotic inflammation/infection in patients with the diagnosis of clinical chorioamnionitis at term;

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and 2) compare the diagnostic performance of a rapid MMP-8 test to that of a conventional enzyme-linked immunosorbent assay (ELISA) interleukin (IL)-6 test for patients with clinical chorioamnionitis at term.

Materials and Methods—A retrospective cohort study was conducted. A transabdominal amniocentesis was performed in patients with clinical chorioamnionitis at term (n=44). Amniotic fluid was analyzed using cultivation techniques (for aerobic and anaerobic bacteria as well as genital Mycoplasmas) and broad-range polymerase chain reaction (PCR) coupled with electrospray ionization mass spectrometry (PCR/ESI-MS). Amniotic fluid IL-6 concentrations were determined by ELISA, and rapid MMP-8 results were determined by Yoon's MMP-8 Check®. Intra-amniotic inflammation was defined as an elevated amniotic fluid IL-6 concentration ≥ 2.6 ng/mL, and intra-amniotic infection was diagnosed by the presence of microorganisms in the amniotic fluid accompanied by intra-amniotic inflammation. The diagnostic indices of Yoon's MMP-8 Check® for the identification of intra-amniotic inflammation were calculated. In order to objectively compare Yoon's MMP-8 Check® to the ELISA IL-6 test for the identification of intra-amniotic inflammation, we used an amniotic fluid white blood cell (WBC) count ≥ 50 cells/mm³ to define intra-amniotic inflammation.

Results—1) A positive rapid MMP-8 test had a sensitivity of 82.4% (28/34), specificity of 90% (9/10), positive predictive value of 96.6% (28/29), negative predictive value of 60% (9/15), positive likelihood ratio 8.2 (95% CI 1.3-53.2), and negative likelihood ratio 0.2 (95% CI 0.1-0.4) for the identification of intra-amniotic inflammation (prevalence 77.3%); 2) a positive rapid MMP-8 test had a sensitivity of 91.7% (22/24), specificity of 65% (13/20), positive predictive value of 75.9% (22/29), negative predictive value of 86.7% (13/15), positive likelihood of ratio 2.6 (95% CI 1.4-4.8), and negative likelihood ratio 0.1 (95% CI 0.03-0.5) for the identification of intra-amniotic infection; 3) the rapid MMP-8 test had a significantly higher specificity than the ELISA IL-6 test in the identification of intra-amniotic inflammation as determined by an amniotic fluid WBC count ≥ 50 cells/mm³. The sensitivity and accuracy of the rapid MMP-8 test were comparable to those of the ELISA IL-6 test; and 4) importantly, the rapid MMP-8 test had 100% sensitivity and 100% negative predictive value in the identification of neonates affected with fetal inflammatory response syndrome (FIRS).

Conclusion—The rapid diagnosis of intra-amniotic inflammation is possible by analysis of amniotic fluid using a point-of-care test for MMP-8. Patients with a positive test are at risk for delivering a neonate affected with systemic inflammation, a risk factor for adverse neonatal outcome.

Keywords

amniocentesis; amniotic fluid; biomarkers; interleukin-6 (IL-6); matrix metalloproteinase (MMP-8); microbial invasion of the amniotic cavity (MIAC); point-of-care test; pregnancy

Introduction

Clinical chorioamnionitis is the most common infection/inflammatory process diagnosed in labor and delivery units worldwide (1-9). The condition is a syndrome (10) that can be caused by: 1) intra-amniotic infection (10, 11); 2) intra-amniotic inflammation without demonstrable microorganisms (i.e., sterile intra-amniotic inflammation) (10); and 3)

maternal systemic inflammation that is not associated with intra-amniotic inflammation (12). The presence of intraamniotic inflammation is a risk factor for adverse maternal (5, 13-18) and neonatal (19-36) outcomes in a broad range of obstetrical syndromes (20, 37-44), including clinical chorioamnionitis at term (10, 45). Although the diagnosis of intra-amniotic infection has relied on culture results (46-48), such information is not immediately available for patient management. Therefore, the diagnosis of intra-amniotic inflammation could be helpful as a proxy for intraamniotic infection while results of microbiologic studies are pending. A rapid test is now available for the diagnosis of intra-amniotic inflammation based on the determination of neutrophil collagenase or matrix metalloproteinase-8 (MMP-8).

Amniotic fluid MMP-8 concentration has diagnostic and prognostic value in the identification of intra-amniotic inflammation (49, 50), intra-amniotic infection (50-55), acute inflammatory lesions of the placenta, especially funisitis (56, 57), imminent spontaneous preterm birth (49, 52, 53), and adverse neonatal outcome such as cerebral palsy (49-54, 57-65). However, the determination of MMP-8 using a conventional enzyme-linked immunosorbent assay (ELISA) test is time-consuming, and the results are often not available in time to guide management and clinical decisions for suspected cases of intra-amniotic inflammation.

A rapid MMP-8 test has recently been developed and provides accurate results without the need for costly instruments (49, 50, 57, 64-68). Previous studies reported that the rapid MMP-8 test results strongly correlate with those of conventional MMP-8 ELISA tests (49) and have diagnostic as well as prognostic value in the identification of impending preterm delivery in patients who have preterm labor and intact membranes (49, 66), preterm prelabor rupture of the membranes (preterm PROM) (50, 64), funisitis in preterm delivery (57), microbial invasion of the amniotic cavity (MIAC) (55), and early spontaneous preterm delivery in asymptomatic midtrimester patients (65). Nonetheless, there is no information about the diagnostic value of the amniotic fluid rapid MMP-8 test to identify intra-amniotic inflammation in cases of clinical chorioamnionitis at term.

Therefore, the objectives of this study were to: 1) evaluate the diagnostic indices of a rapid MMP-8 test for the identification of intra-amniotic inflammation/infection in patients with the diagnosis of clinical chorioamnionitis at term; and 2) compare the diagnostic performance of a rapid MMP-8 test to that of a conventional ELISA interleukin (IL)-6 test for patients with clinical chorioamnionitis at term.

Materials and Methods

Study population

This retrospective cohort study was conducted with patients who had clinical chorioamnionitis at term for whom samples of amniotic fluid were available in the Bank of Biological Materials at Wayne State University, the Detroit Medical Center, and the Perinatology Research Branch of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). Inclusion criteria were: 1) singleton gestation; 2) gestational age $\geq$ 37 weeks; and 3) transabdominal amniocentesis performed for molecular

microbiologic studies. Exclusion criteria included multiple gestations or fetal malformations. The samples were collected from patients cared for at the Sótero del Río Hospital, a major affiliate of the Catholic University in Santiago, Chile. Patients enrolled in this study comprised a subset of those who had been included in previous studies (8, 10, 12, 36, 45, 69). All amniocenteses were performed for clinical indications at the discretion of the treating physician(s).

Maternal and neonatal data were obtained from clinical chart reviews. Women with the diagnosis of clinical chorioamnionitis were counseled to undergo an amniocentesis. Written informed consent to donate additional amniotic fluid and to collect clinical information for research purposes was obtained from each participant. The Institutional Review Boards of Wayne State University, NICHD, and Sótero del Río Hospital approved the use of samples and biological specimens as well as the use of clinical data for research purposes.

Clinical definitions

Clinical chorioamnionitis was diagnosed using the criteria proposed by Gibbs et al. (1): the presence of maternal fever (temperature $>37.8^{\circ}\text{C}$) accompanied by two or more of the following: 1) maternal tachycardia (heart rate >100 beats/min); 2) uterine tenderness; 3) foul-smelling amniotic fluid; 4) fetal tachycardia (heart rate >160 beats/min); and 5) maternal leukocytosis (leukocyte count $>15,000$ cells/ mm^3) (1-4, 6, 11, 70-73).

The presence of intra-amniotic inflammation was defined as an elevated amniotic fluid IL-6 concentration 2.6 ng/mL (20, 28, 29, 74-79). To compare the diagnostic performance of Yoon's MMP-8 Check® (SK Pharma Co., Ltd., Kyunggi-do, Republic of Korea) and that of a conventional ELISA IL-6 test (Human IL-6 Quantikine ELISA kit, D6050, R&D Systems, Minneapolis, MN, USA), we defined intra-amniotic inflammation as an amniotic fluid white blood cell (WBC) count 50 cells/ mm^3 (80). Intra-amniotic infection (microbial-associated intraamniotic inflammation) was diagnosed in the presence of both intra-amniotic inflammation and microorganisms detected in the amniotic fluid using cultivation or molecular microbiologic techniques (PCR/ESI-MS; Ibis® Technology, Athogen, Carlsbad, CA, USA) (81-83).

Acute inflammatory lesions of the placenta included acute histologic chorioamnionitis and acute funisitis (84, 85). Acute histologic chorioamnionitis was diagnosed based on the infiltration of inflammatory cells (neutrophils) in the chorionic plate and/or chorioamniotic membranes (20, 84-86). Acute funisitis was diagnosed based on the infiltration of neutrophils in the walls of the umbilical vessels and/or in the Wharton's jelly, also using previously reported criteria (84-87). Fetal inflammatory response syndrome (FIRS) was defined as an elevation of an umbilical cord plasma IL-6 concentration >11 pg/mL (87-92).

Sample collection, amniotic fluid processing, amniotic fluid analysis for microbiologic studies, IL-6 concentrations (10), and the performance and interpretation of Yoon's MMP-8 Check® (49, 50, 57, 65, 93) were previously described.

Study outcomes

The primary outcomes were the diagnostic indices, predictive values, and likelihood ratios of Yoon's MMP-8 Check® for the identification of intra-amniotic inflammation (defined as an amniotic fluid ELISA IL-6 concentration ≥ 2.6 ng/mL). The secondary outcomes were the diagnostic indices, predictive values, and likelihood ratios of Yoon's MMP-8 Check® for the identification of intra-amniotic infection, the presence of acute inflammatory histologic features of the placenta (acute histologic chorioamnionitis and/or funisitis), and FIRS.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess normality of arithmetic data distributions. Diagnostic indices, predictive values, and likelihood ratios were calculated to identify each outcome. Comparisons of sensitivity, and specificity, between Yoon's MMP-8 Check® and the ELISA IL-6 test were performed using McNemar's test. A p-value of <0.05 indicated statistical significance. All p-values were determined from a two-sided test. Statistical analysis was performed using SPSS Version 19 (IBM Corp, Armonk, NY, USA) and the R statistical language and environment (94).

Results

Characteristics of the study population

A total of 44 patients who had clinical chorioamnionitis at term [39.6 (IQR: 38.8-40.7) weeks of gestation] were included in this study. Table 1 displays the clinical characteristics of the study population. The overall rate of intra-amniotic inflammation was 77.3% (34/44). The rate of intra-amniotic infection was 54.5% (24/44) and that of intra-amniotic inflammation without detectable bacteria was 22.7% (10/44). The most common microorganisms isolated from the amniotic fluid were *Gardnerella vaginalis* (n=10) and *Ureaplasma urealyticum* (n=8), and 66% (16/24) had a polymicrobial infection. Acute histologic chorioamnionitis and acute funisitis were found in 46.5% (20/43) and 30.2% (13/43) of cases, respectively. The prevalence of FIRS was 23.3% (10/43). This information has been extensively covered in prior publications (8, 10, 12, 36, 45, 69).

Performance of the rapid MMP-8 test to identify intra-amniotic inflammation, intra-amniotic infection, acute inflammatory lesions of the placenta, and neonates with FIRS

The diagnostic indices, predictive values, and likelihood ratios of the rapid MMP-8 test for different outcomes of interest are displayed in Table 2. The sensitivity, specificity, and positive and negative likelihood ratios of the rapid MMP-8 test for the identification of intraamniotic inflammation were 82.4% (28/34), 90% (9/10), 8.2, and 0.2, respectively. The positive and negative predictive values were 96.6% (28/29) and 60% (9/15), respectively, in a population with a prevalence of 77.3% (34/44).

This test also demonstrated a high sensitivity [91.7% (22/24)] and negative predictive value [86.7% (13/15)] for the identification of intra-amniotic infection. Importantly, the rapid MMP-8 test had a 100% (10/10) sensitivity and a 100% (14/14) negative predictive value for the identification of mothers who subsequently delivered neonates with FIRS.

Comparison of the diagnostic performances between the rapid MMP-8 test and the ELISA IL-6 test

The prevalence of intra-amniotic inflammation (defined as a WBC count ≥ 50 cells/mm³) and intra-amniotic infection was 52.3% (23/44) and 54.5% (24/44), respectively (Table 1). The rapid MMP-8 test had a significantly higher specificity than the conventional ELISA IL-6 test in the identification of intra-amniotic inflammation [66.7% (14/21) vs. 42.9% (9/21); $p=0.03$]. The accuracy of the rapid MMP-8 kit was 81.8% (36/44) and that of the IL-6 ELISA was 70.5% (31/44) (Table 2). In addition, both tests had comparable performances for the identification of intra-amniotic infection, acute inflammatory lesions of the placenta, acute funisitis, and the presence of FIRS in neonates, as the 95% confidence intervals (CI) overlapped (Table 2). Similar results were obtained when intra-amniotic inflammation was defined according to the amniotic fluid WBC counts (> 50 cells/mm³) (Table 3). Patients with discrepant results between the rapid MMP-8 and the IL-6 ELISA tests are described in the Supplementary Table.

Discussion

Principal findings of this study

In the context of clinical chorioamnionitis at term, 1) the positive rapid MMP-8 test had a sensitivity of 82.4% (28/34), specificity of 90% (9/10), positive predictive value of 96.6% (28/29), negative predictive value of 60% (9/15), positive likelihood ratio of 8.2 (95% CI, 1.3-53.2), and negative likelihood ratio of 0.2 (95% CI, 0.1-0.4) for the identification of intraamniotic inflammation (prevalence, 77.3%); 2) the positive rapid MMP-8 test had a sensitivity of 91.7% (22/24), specificity of 65% (13/20), positive predictive value of 75.9% (22/29), negative predictive value of 86.7% (13/15), positive likelihood ratio of 2.6 (95% CI, 1.4-4.8), and negative likelihood ratio of 0.1 (95% CI, 0.03-0.5) for the identification of intra-amniotic infection; 3) the rapid MMP-8 test had a significantly higher specificity than the ELISA IL-6 test in the identification of intra-amniotic inflammation as determined by an amniotic fluid WBC count ≥ 50 cells/mm³. The sensitivity and accuracy of the rapid MMP-8 test were comparable to those of the ELISA IL-6 test; and 4) importantly, the rapid MMP-8 test had a 100% sensitivity and 100% negative predictive value in the identification of neonates affected with FIRS.

Clinical chorioamnionitis at term: the need for a rapid test to identify intra-amniotic inflammation/infection—Clinical chorioamnionitis is the most common infection diagnosed in labor and delivery units (4-7). This condition is associated with substantial maternal and neonatal morbidity, including maternal sepsis (95), dysfunctional labor (16, 96), postpartum endomyometritis (15) and hemorrhage (5, 96), early-onset neonatal sepsis (19, 95, 97-99), and cerebral palsy (100-103). Currently, the diagnosis of clinical chorioamnionitis is based on maternal clinical signs that have only about a 50% accuracy for the identification of proven intra-amniotic infection (8). The management of mothers with suspected clinical chorioamnionitis includes treatment with antimicrobial agents (47, 71, 104-106) and labor augmentation (4, 6, 73, 107). Neonatologists often perform a laboratory work-up for sepsis and initiate antibiotic treatment (108-114).

Interestingly, a fraction of patients with clinical chorioamnionitis at term had proven intra-amniotic infection (10), but others had intra-amniotic inflammation without bacteria or without intra-amniotic inflammation. Only one-fifth (21%) of neonates born to mothers with clinical chorioamnionitis at term had FIRS (36). In our studies, all neonates with FIRS were born to mothers with clinical chorioamnionitis who also had intra-amniotic infection (36).

Patients with intra-amniotic inflammation without microorganisms or without intraamniotic inflammation may not benefit from the administration of antibiotics, which is the standard of practice. In general, the administration of antimicrobial agents in this context has been considered to have little, if any, adverse events. However, recent evidence suggests that antibiotic administration can have adverse maternal and neonatal effects. For example, antibiotic administration is the most common cause of maternal anaphylaxis (115-118); although this condition is rare (prevalence of 2.7 cases/100,000 deliveries) (118), it can lead to maternal death and fetal compromise, including neurological damage (119-121). In addition, excessive antibiotic use increases the development of antibiotic-resistant microorganisms (122-124).

For newborns, consequences from prenatal exposure to antibiotics include: 1) the need for a sepsis work-up and other laboratory testing, which is associated with increased workload and costs (108-114); 2) a prolonged hospital stay that can predispose these neonates to acquire infections caused by multi-drug-resistant bacteria (110, 112); 3) the separation of newborns from parents with the potential for impaired mother-neonate bonding and breastfeeding; and 4) the alteration of gut microbiota (125-128). The change in neonatal gut microbiota due to intrapartum antibiotic exposure is currently a subject of intense investigation. For example, it has been shown that prenatal exposure to antimicrobial agents is associated with a reduction in *Bacteroides* and *Parabacteroides* species and an over-representation of *Enterococcus* and *Clostridium* species within the neonatal gut microbial community (127). Importantly, the disturbance of neonatal gut microbiota can persist until 12 months of age after birth (127) and appears to have short- and long-term effects on the immune response. The latter includes an increased predisposition to the development of allergies (129-131), asthma (132-134), atopic disease (135-137), obesity (138-140), and neonatal sepsis (141-143). The imbalance in the composition of the neonatal gut microbial community in mice due to perinatal antibiotic administration has been shown to prevent granulocytosis and to reduce circulating neutrophils one to three days after birth, thus increasing the susceptibility to blood-borne infections and subsequent development of sepsis (144, 145).

There is mounting evidence indicating that a subset of women with clinical chorioamnionitis at term and their neonates requires intrapartum antibiotics and that a subgroup may not need such treatment. Thus far, clinical signs (8), inflammatory-related proteins in the maternal (12) or cord (36) blood, or placental histopathology (69) have limited value for the identification of intra-amniotic infection/inflammation in patients who have clinical chorioamnionitis at term. The results of several studies in this population suggest that amniotic fluid analysis may help to distinguish between patients with or without intra-amniotic inflammation/infection and to guide clinical decisions. Although amniocentesis is an invasive procedure, transcervical collection of amniotic fluid is now possible with a

simple-to-use device now available for clinical use (146). The optimal method to diagnose intra-amniotic inflammation in fluid retrieved by this device is a subject of current investigation. However, if biomarkers that allow the diagnosis of intra-amniotic inflammation with such biological material are identifiable, then the diagnosis of intra-amniotic inflammation could be made non-invasively.

Rapid MMP-8 test of amniotic fluid—The rapid MMP-8 test of amniotic fluid fulfills the standard requirements for a point-of-care test as results are available in a timely fashion: the test is simple to use, sensitive, inexpensive, and operator- and instrument-independent. Previous studies reported that the rapid MMP-8 test can be used at the bedside to identify intra-amniotic inflammation in patients at risk of imminent preterm delivery (49, 93), preterm PROM (50, 64), funisitis (57), and MIAC (55). Moreover, the diagnostic performance of the rapid MMP-8 test for the identification of intraamniotic inflammation is equivalent to that of a conventional immunoassay for MMP-8 (49). The results of the present study confirm the value of the rapid MMP-8 test for the determination of intra-amniotic inflammation in patients with clinical chorioamnionitis at term.

Given the high positive predictive value and positive likelihood ratio of the rapid MMP-8 test, a positive MMP-8 result can be interpreted as evidence of intra-amniotic inflammation. We propose that the next step in management should be differentiation between sterile and microbial-associated intra-amniotic inflammation; this will determine whether anti-microbial or anti-inflammatory treatment needs to be administered. This can be achieved by sequencing the 16S rRNA gene using novel molecular microbiologic techniques that will allow the rapid detection of microbial signatures (81-83).

The rapid MMP-8 test had a sensitivity of 91.7% (22/24) and a negative predictive value of 86.7% (13/15) for the identification of intra-amniotic infection. Due to the high negative predictive value, a negative result can help to identify women with clinical chorioamnionitis at term who *are not* at risk for intra-amniotic infection, and this information can be used to decrease the index of suspicion of proven intra-amniotic infection, therefore preventing the overuse of anti-microbial agents.

Importantly, the rapid MMP-8 test demonstrated a 100% (10/10) sensitivity and a 100% (14/14) negative predictive value for the identification of FIRS. As neonates with this condition are at an increased risk of neonatal sepsis, antimicrobial treatment should be promptly initiated. Our results indicated that a positive amniotic fluid rapid MMP-8 test can identify most neonates with FIRS, and a negative test result would exclude the presence of FIRS in neonates born to mothers with clinical chorioamnionitis at term. Collectively, the rapid MMP-8 test has optimal characteristics as a point-of-care test.

Strengths and limitations—The strengths of the study include the participation of a homogeneous group of patients with clinical chorioamnionitis at term, the use of both cultivation and molecular microbiologic techniques for the identification of microorganisms, and treatment not influenced by the rapid MMP-8 test. We acknowledge that the major limitation is the sample size; however, this is a unique population in which characterizations of intra-amniotic inflammation and intra-amniotic infection were performed by

amniocentesis, a procedure infrequently used in the identification of patients with clinical chorioamnionitis at term. This procedure was implemented at the Stero del Ro Hospital because of the need to address large-scale use of antibiotics and the performance of a high number of septic work-ups of patients with maternal fever. The samples employed in this study have been used for other studies of amniotic fluid, including the amniotic fluid lipidome (147) and to characterize the nature of the inflammatory response. Therefore, replication of these findings in a separate population is warranted.

Conclusions

A rapid MMP-8 point-of-care test can be used for the diagnosis of intra-amniotic inflammation and to identify the mother at risk for delivering a neonate with systemic inflammation among patients with clinical chorioamnionitis at term.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1
Clinical characteristics of the study population

Characteristics	Median (interquartile range) or percent (n=44)
Maternal age (years)	21 (18-25)
Body mass index (kg/m ²)	23.7 (21.6-24.6)
Nulliparity (%)	65.9% (29/44)
Smoking (%)	11.4% (5/44)
Rupture of the membranes at the time of amniocentesis (%)	65.9% (29/44)
Gestational age at delivery (weeks)	39.6 (38.8-40.7)
Labor (%)	
- Spontaneous	84.1% (37/44)
- Induced	15.9% (7/44)
Birthweight (grams)	3510 (3220-3772)
Amniotic fluid white blood cells (cells/mm ³)	66.5 (5.0-648.8)
Amniotic fluid glucose (mg/dL)	9.0 (9.0-9.0)
Amniotic fluid ELISA IL-6 (ng/mL)	5.7 (2.9-18.3)
Positive amniotic fluid Gram stain (%)	11.4% (5/44)
Intra-amniotic inflammation (ELISA IL-6 ≥ 2.6 ng/mL) (%)	77.3% (34/44)
Sterile intra-amniotic inflammation (%)	22.7% (10/44)
Intra-amniotic infection (%)	54.5% (24/44)
Amniotic fluid white blood cell count ≥ 50 cells/mm ³ (%)	52.3% (23/44)
Acute inflammatory lesions of the placenta (%) *	48.8% (21/43)
Acute histologic chorioamnionitis (%) *	46.5% (20/43)
Acute funisitis (%) *	30.2% (13/43)
Fetal inflammatory response syndrome (FIRS) (%) *	23.3% (10/43)

Data presented as median (interquartile range) or % (n).

ELISA, enzyme-linked immunosorbent assay; IL, Interleukin;

Acute inflammatory lesions of the placenta include acute histologic chorioamnionitis and/or acute funisitis.

* Among these patients, a placental histology report was not available for one patient and the fetal plasma IL-6 concentration was not available for one patient.

Table 2
Diagnostic performance of point-of-care rapid MMP-8 and ELISA IL-6 tests for the identification of intra-amniotic inflammation/infection, placental lesions consistent with acute inflammation, and fetal inflammatory response syndrome

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		ELISA IL-6	
		% (n)	95% CI	% (n)	95% CI
Intra-amniotic inflammation [77.3% (34/44)]	Sensitivity	82.4% (28/34)	65.5-93.2		
	Specificity	90% (9/10)	55.5-99.8		
	Positive Likelihood Ratio	8.2	1.3-53.2		
	Negative Likelihood Ratio	0.2	0.1-0.4		
	Positive predictive value	96.6% (28/29)	82.2-99.9		
	Negative predictive value	60% (9/15)	32.3-83.7		
	Accuracy	84.1% (37/44)	-		
Intra-amniotic infection [54.5% (24/44)]	Sensitivity	91.7% (22/24)	73.0-98.9	100% (24/24)	85.8-100
	Specificity	65% (13/20)	40.8-84.6	50% (10/20)	27.2-72.8
	Positive Likelihood Ratio	2.6	1.4-4.8	2.0	1.3-3.1
	Negative Likelihood Ratio	0.1	0.03-0.5	0	-
	Positive predictive value	75.9% (22/29)	56.5-89.7	70.6% (24/34)	52.5-84.9
	Negative predictive value	86.7% (13/15)	59.5-98.3	100% (10/10)	69.2-100
	Accuracy	79.5% (35/44)	-	77.3% (34/44)	-
Acute inflammatory lesions of the placenta [48.8% (21/43)]	Sensitivity	85.7% (18/21)	63.7-96.9	90.5% (19/21)	69.6-98.8
	Specificity	54.5% (12/22)	32.2-75.6	36.4% (8/22)	17.2-59.3
	Positive Likelihood Ratio	1.9	1.2-3.1	1.4	1.0-2.0
	Negative Likelihood Ratio	0.3	0.1-0.8	0.3	0.1-1.0
	Positive predictive value	64.3% (18/28)	44.1-81.4	57.6% (19/33)	39.2-74.5
	Negative predictive value	80% (12/15)	51.9-95.7	80% (8/10)	44.4-97.5
	Accuracy	69.8% (30/43)	-	62.8% (27/43)	-
Acute funisitis [30.2% (13/43)]	Sensitivity	84.6% (11/13)	54.6-98.1	84.6% (11/13)	54.6-98.1
	Specificity	43.3% (13/30)	25.5-62.6	26.7% (8/30)	12.3-45.9
	Positive Likelihood Ratio	1.5	1.0-2.2	1.2	0.8-1.6
	Negative Likelihood Ratio	0.4	0.1-1.4	0.6	0.1-2.4

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		ELISA IL-6	
		% (n)	95% CI	% (n)	95% CI
	Positive predictive value	39.3% (11/28)	21.5-59.4	33.3% (11/33)	17.9-51.8
	Negative predictive value	86.7% (13/15)	59.5-98.3	80% (8/10)	44.4-97.5
	Accuracy	55.8% (24/43)	-	44.2% (19/43)	-
Fetal inflammatory response syndrome [23.3% (10/43)]	Sensitivity	100% (10/10)	69.2-100	100% (10/10)	69.2-100
	Specificity	42.4% (14/33)	25.5-60.8	30.3% (10/33)	15.6-48.7
	Positive Likelihood Ratio	1.7	1.3-2.3	1.4	1.2-1.8
	Negative Likelihood Ratio	0	-	0	-
	Positive predictive value	34.5% (10/29)	17.9-54.3	30.3% (10/33)	15.6-48.7
	Negative predictive value	100% (14/14)	76.8-100	100% (10/10)	69.2-100
	Accuracy	55.8% (24/43)	-	46.5% (20/43)	-

ELISA: enzyme-linked immunosorbent assay; IL: interleukin; MMP: matrix metalloproteinase.

Table 3

Diagnostic performances of point-of-care rapid MMP-8 and ELISA IL-6 tests for the identification of intra-amniotic inflammation and infection determined by using an amniotic fluid white blood cell count

Outcomes and Prevalence % (n)	Diagnostic Performance	Rapid MMP-8		ELISA IL-6	
		% (n)	95% CI	% (n)	95% CI
Intra-amniotic inflammation identified by AF WBC count 50 cells/mm ³ [52.3% (23/44)]	Sensitivity	95.7% (22/23)	78.1-99.9	95.7% (22/23)	78.1-99.9
	Specificity *	66.7% (14/21)	43.0-85.4	42.9% (9/21)	21.8-66.0
	Positive Likelihood Ratio	2.9	1.6-5.3	1.7	1.1-2.5
	Negative Likelihood Ratio	0.07	0.01-0.5	0.1	0.01-0.7
	Positive predictive value	75.9% (22/29)	56.5-89.7	64.7% (22/34)	46.5-80.3
	Negative predictive value	93.3% (14/15)	68.1-99.8	90.0% (9/10)	55.5-99.8
	Accuracy	81.8% (36/44)	-	70.5% (31/44)	-
Intra-amniotic infection identified by a positive AF culture and an AF WBC count 50 cells/mm ³ [40.9% (18/44)]	Sensitivity	100% (18/18)	81.5-100	100% (18/18)	81.5-100
	Specificity **	57.7% (15/26)	36.9-76.7	38.5% (10/26)	20.2-59.4
	Positive Likelihood Ratio	2.4	1.5-3.7	1.6	1.2-2.2
	Negative Likelihood Ratio	0	-	0	-
	Positive predictive value	62.1% (18/29)	42.3-79.3	52.9% (18/34)	35.1-70.2
	Negative predictive value	100% (15/15)	78.2-100	100% (10/10)	69.2-100
	Accuracy	75% (33/44)	-	63.6% (28/44)	-

* p-value=0.03,

** p-value=0.058: a comparison between the rapid MMP-8 test and the ELISA IL-6 test using the McNemar's test.